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**BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES**

Application Number: 08/468,610
Filing Date: June 06, 1995
Appellant(s): BURTON ET AL.

Jeffrey A. McKinney
For Appellant

**EXAMINER'S ANSWER**

This is in response to the appeal brief filed June 4, 2004.

***(1) Real Party in Interest***

A statement identifying the real party in interest is contained in the brief.

***(2) Related Appeals and Interferences***

A statement identifying the related appeals and interferences which will directly affect or be directly affected by or have a bearing on the decision in the pending appeal is contained in the brief.

***(3) Status of Claims***

The statement of the status of the claims contained in the brief is correct.

***(4) Status of Amendments After Final***

The appellant's statement of the status of amendments after final rejection contained in the brief is correct. Note that the after final amendment filed on June 4, 2004, concurrently with the appeal brief, has been entered.

***(5) Summary of Invention***

The summary of invention contained in the brief is correct.

***(6) Issues***

The appellant's statement of the issues in the brief is correct.

***(7) Grouping of Claims***

Appellant's brief includes a statement that the claims do not stand or fall together and provides reasons as set forth in 37 CFR 1.192(c)(7) and (c)(8).

***(8) Claims Appealed***

A substantially correct copy of appealed claim 56 appears on pages 4 and 5 of Appendix "A" to the appellant's brief. The minor errors are as follows:

The recitation "hydroxyl groups" is still present in claim 56, despite the deletion of that term in the after final amendment filed June 4, 2004.

***(9) Prior Art of Record***

Boardman et al., (1953) "Separation of Neutral Proteins on Ion-Exchange Resins", **Nature**, vol. 171, 208-210.

Sasaki et al. (1979) "Hydrophobic-Ionic Chromatography, Its Application to Purification of Porcine Pancreas Enzymes", **J. Biochem.**, vol. 86, pages 1537-1548.

Sasaki et al. (1982) "Hydrophobic-Ionic Chromatography, Its Application to Microbial Glucose Oxidase, Hyaluronidase, Cholesterol Oxidase, and Cholesterol Esterase", **J. Biochem.**, vol. 91, pages 1555-1561.

Kunin, R., (1958) "Ion Exchange Resins", Wiley Interscience, New York, pages 34-39.

Topp et al., (1949) "Properties of Ion-Exchange Resins in Relation to their Structure. Part 1. Titration Curves", **J. Chem. Soc. Pt. 2**, 3299-3303.

Kitchener, J.A., (1957) "Properties and Behavior, 5. Effect of pH on Exchange Equilibria" in ***Ion Exchangers in Organic and Biochemistry***, eds. Calmon et al., Wiley Interscience, New York, pages 63-65.

Guthrie, J.D., (1957) "Ion Exchangers of Plant Origin, G. Evaluation of Capacity and Strength", in ***Ion Exchangers in Organic and Biochemistry***, eds. Calmon et al., Wiley Interscience, New York, pages 558-559.

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|---|---|---|
| 4,401,629 | HANCOCK et al | 8-1983 |
| JP 1-211543 | KITAMURA et al | 8-1989 |
| JP 61-33130 | KONDO et al | 2-1986 |
| 4,950,807 | IIMURO et al | 8-1990 |
| 4,810,391 | BRUEGGER | 3-1989 |
| 3,835,072 | ECONOMY et al | 9-1974 |
| 4,154,676 | JONES et al | 5-1979 |
| JP 60-137441 | TOKUYAMA et al | 7-1985 |

***(10) Grounds of Rejection***

The following ground(s) of rejection are applicable to the appealed claims:

Claims 1-2, 4-5, 10-16, 18, 20 and 22-23 stand rejected under 35 U.S.C. 102(b) as being anticipated by Boardman et al. (1953).

A complex between an ion exchange resin and a target protein is claimed wherein the complex is formed at a pH value of between 5-9, where the resin is uncharged, and the target protein is bound to the resin by hydrophobic interactions. The ion exchange resin consists of a solid support matrix and a covalently attached ionizable ligand.

Boardman et al. (1953) disclose separation of proteins on ion exchange media with pH elution. At low pH the cation exchange media is uncharged and binds the proteins. As the pH is raised, the protein is eluted. Figure 1(a) illustrates the technique with cytochrome C on Amberlite IRC-50 [a cross-linked poly(methacrylic acid) with a capacity of 10 Meq/g]. At a pH value of 5, cytochrome C is tightly bound to the media whose carboxylic groups are said to be wholly uncharged. See page 210, left column, first full paragraph ("On the other hand, as is shown in Fig. 1*a*, at pH 5 the carboxylic groups of the resin are almost wholly undissociated… ."). Between pH values of 6-7 the protein elutes from the column. At the same pH range, the affinity for sodium ions increases, corresponding to an ionization of the carboxylic groups on the resin.

Claims 1-5, 7-23 and 55-56 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Boardman et al. (1953),

Sasaki et al. (1979) and Sasaki et al. (1982) in view of Kunin (1958), Topp et al. (1949), Kitchener (1957) and Guthrie (1957) and further in view of Hancock et al. (US 4,401,629), Kitamura et al. (JP 01211543), Tokuyama (JP 60137441), Kondo et al. (JP 61033130), Iimuro et al. (US 4,950,807), Bruegger (US 4,810,391), Economy et al. (US 3,835,072), Jones et al. (US 4,154,676).

A complex between an ion exchange resin and a target protein is claimed wherein the complex is formed at a pH value of between 5-9, where the resin is uncharged, and the target protein is bound to the resin by hydrophobic interactions. The ion exchange resin consists of a solid support matrix and a covalently attached ionizable ligand. The resin may further comprise non-ionizable ligands.

The teachings of Boardman et al. (1953) have been discussed above. Boardman et al. (1953) lack the full range of resins that may be used.

Sasaki et al. (1979) disclose binding several enzymes onto Amberlite CG-50 at a pH value of 4.0 where the carboxyl groups are not dissociated and, consequently, the Amberlite is uncharged. The resin can be eluted by increasing the pH so that the carboxyl groups dissociate with a concomitant loss of hydrophobicity and acquisition of a repulsive charge which in

combination decreases the binding affinity of the bound enzymes. This process of using the Amberlite ion-exchange medium is termed hydrophobic-ionic chromatography. At page 1548, the hydrophobic-ionic type of chromatography is defined as when the order of elution of proteins from the resin "is controlled by the remaining hydrophobic affinity plus the increased electrostatic affinity minus the increased electrostatic repulsion produced as the carboxyl groups are dissociated". Contrary to conventional ion exchange chromatography, enzymes bind to the uncharged functional groups and dissociate when the functional groups become charged.

The acid base titration curve of Amberlite CG-50 shown in Fig. 1 demonstrates that below a pH value of about 4.5, the resin is fully protonated and therefore should exhibit no charge. Additionally, this figure demonstrates to a person of skill in the art how to determine the effective pH range of a given resin in the hydrophobic-ionic chromatography method. That is, the titration curve shows conditions when the protein will be bound by hydrophobic effects, and conditions when ionic effects will dissociate the protein.

The lack of ionic strength dependence on the binding of proteins at pH 4 to Amberlite CG-50 (page 1540, column 2) and elution of proteins with organic solvents (page 1546, column 2)

is further evidence that the enzymes are bound to the ion exchange matrix by hydrophobic effects. Sasaki et al. (1979) lack forming the complex with a resin that is uncharged between pH values of 5-9.

Sasaki et al. (1982) disclose binding several microbial enzymes onto Amberlite CG-50 at a pH value of 4.0 where the carboxyl groups are not dissociated and, consequently, the Amberlite is uncharged. Subsequently, elution is effected by increasing pH to ionize the resin. This overall process is termed hydrophobic-ionic chromatography (abstract). In Figure 5 a cartoon is provided to explain the proposed mechanism of hydrophobic-ionic chromatography. The cartoon clearly indicates, in general terms, that with an acidic group, binding occurs below a certain pH and desorption occurs above the critical pH. Different proteins having different interacting groups are released at different critical pH values (X or Y). This cartoon does not require any particular resin. It is a generalization of the concept of hydrophobic-ionic chromatography. The figure legend to Figure 5 clearly states, "in the case of Amberlite CG-50, X is 4.5". The figure legend continues to describe general mechanism, "with the use of appropriate adsorbent carrying alkaline groups, ... the relationship to pH would be opposite". While the cartoon illustrates the general principle with an ion-

exchange resin that is acidic in nature, if the general mechanism is applied to an ion-exchange resin which is basic in nature, the effect of pH would be the opposite, i.e., lowering the pH would effect elution as the basic resin became charged. To understand this better, consider a resin with an amine functional group attached thereto. At sufficiently alkaline pH values, the resin is in the form of free amine and is uncharged. At lower pH values the basic amine becomes protonated to its conjugate acid and assumes a positive charge. Sasaki et al. (1982) lack forming the complex with a resin that is uncharged between pH values of 5-9.

Kunin (1958) discloses titration curves of several ion exchange media and develops some of the mathematics of describing the dissociation. The well-known Henderson-Hasselbach equation is said to fit the titration data well. Accordingly, the pKa is the pH at which the ionizable group is half titrated. Figure 13 provides titration curves for Amberlite IRC-50 (used by Boardman et al., 1953) in water and in different concentrations of KCl. The pKa in water is about 8.5 and lower in the presence of KCl. At 2 pH units below the pKa, the ionized form of an acid comprises less than 0.1% of the total acid.

Topp et al. (1949) discloses the titration of several cases of ion exchange resins. Figure 2 shows the titration with

poly(methacrylic acid). In the absence of added salt, the pKa is seen to be about 8.5-9, while in the presence of 0.1 M NaCl, the pKa is lowered to about 7. In the absence of salt, exchange does not occur below pH value of 6.

Kitchener (1957) discloses that the carboxylic acid functionality normally titrates between 7 and 11 with a midpoint of about 9 which is lowered to about 7 in the presence of 0.1N KCl (based upon the data of Topp et al., 1949).

Guthrie (1957) lists the pH at half capacity (which corresponds to a phenomenological pKa) for a number of ion exchange cotton fabrics in Table I. Several of the modifying groups have pKa values in the range of 5-9.

A person of ordinary skill in the art at the time the invention was made would have been motivated to use ion exchange media to separate proteins where the proteins are bound at a pH value where the media is uncharged and then eluting by changing the pH to a value where the media is charged according to Boardman et al. (1953), Sasaki et al. (1979) and Sasaki et al. (1982) because media which can be used in the claimed pH range of 5-9 are known in the art as demonstrated by Kunin (1958), Topp et al. (1949), Kitchener (1957) and Guthrie (1957).

A wide range of ion-exchange media is known in the art and dozens of patents have been issued describing them, including

media having the ionizable groups recited in the claims under examination. Hancock et al. (US 4,401,629) disclose polymeric ion exchange resins comprising a cross-linked vinyl backbone with attached imidazolyl groups optionally substituted with pyridyl, imidiazolyl or amino groups (see abstract).

Kitamura et al. (JP 01211543), Kondo et al. (JP 61033130) or Iimuro et al. (US 4,950,807) disclose polymeric ion exchange resins having pyridyl group as the exchange group.

Tokuyama (JP 60137441) or Bruegger (US 4,810,391) disclose ion exchange resins which may have phenolic hydroxyl group as the exchange group.

Economy et al. (US 3,835,072) discloses ion exchange fibers which may have a primary, secondary, tertiary or quaternary amino group as the exchange group (column 1, lines 67-72).

Jones et al. (US 4,154,676) disclose polymeric ion exchange resins having the morpholino (column 2) group *inter alia* as the exchange group.

In sum, Boardman et al. (1953) teach a resin with binding properties in the critical region. Sasaki et al. references were cited because they clearly disclose the concept of the instant invention. The cartoon of Figure 5 in Sasaki et al. (1982) outlines the method in its most general form. The cartoon illustrates an acidic group undergoing ionization. At low pH the

proteins absorb to the uncharged resin by hydrophobic effects. As the pH is raised, the resin becomes charged and the proteins elute. The figure legend goes on to say that the method "can be used with an absorbent carrying alkaline groups, although the relationship to pH would be the opposite". That is, at high pH the resin is uncharged and the proteins bind by hydrophobic effects. As the pH is lowered, the resin becomes charged and the proteins elute. Sasaki et al. only illustrates the method with a resin that binds at pH 4.5. It is clear that their conceptualization only requires that there be resins which bind in the critical region of 5-9.

In Kunin (1958), for example, the titration curves for salt exchange were used to calculate the apparent ionization constants of some acidic resins. These are listed at page 35. In Topp et al. (1949) it is clear, for example, that the carboxyl group of polymethacrylate (Figure 2) undergoes salt exchange in the region of 6-9 in the absence of added salt (this is further discussed at page 3301, first full paragraph). The values for pKa can be compared to the values for pKa for suitable resins given in the instant disclosure on Tables 3 and 4 (alkaline and acid resins respectively). It would appear that suitable resins are relatively easy to obtain. The references clearly lead to using suitable resins in the allegedly critical region of pH.

Such resins are clearly known in the art and reasonably suggested by Hancock et al. (US 4,401,629), Kitamura et al. (JP 01211543), Tokuyama (JP 60137441), Kondo et al. (JP 61033130), Iimuro et al. (US 4,950,807), Bruegger (US 4,810,391), Economy et al. (US 3,835,072), Jones et al. (US 4,154,676). These latter references disclose resins containing ionizable ligands as the exchange groups alleged to be suitable and specifically claimed as such in claims 55-56. Given the teachings of the art of record, it would constitute nothing more than routine optimization to select a suitable ion exchange media compatible with the target protein and having ionizable functional groups in the desired range of 5-9.

Hence, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to separate proteins with ion exchange media with a pKa value in the range of 5-9, as recited in the claims under examination.

***(11) Response to Argument***

Appellant's arguments do not demonstrate error. With respect to the rejection under § 102, appellant urges that Boardman's resin is not uncharged when bound to the protein. It is argued that the Declaration of Becker supports the position that the resin does not meet the claim limitation that the resin is uncharged at the pH where the protein is bound. Becker argues

that the pKa of the resin is 6.1. A figure from the manufacturer's data sheet is provided to support the assertion that the resin supports a partial charge at pH value of 5.0. It is asserted that the Declarations show that at pH 5.0 the resin retains a partial charge of 20%, which is greater than the "less than 5%" as required by the disclosure. It is also urged that the relatively low ionic strength solutions used in the titrations of Boardman teach nothing regarding the resin-protein/peptide complexes at high ionic strength.

The most salient feature of Boardman, the titration curves shown in Figure 1, seems to have been overlooked. Clearly at pH 5.0, cytochrome C is bound, and elutes between pH values of 6-7 or 6-8 depending on ionic strength. Concomitantly, the resin takes up the sodium ions. That is, when the resin is uncharged, no sodium ions are attracted to, or taken up by, the resin.

Fig. 1a of Boardman indicates that, at pH 12, when the resin is 100% ionized, the resin can adsorb a maximum of about 8.8 mg-equivalent sodium ions/gm of dry resin. See vertical scale at right hand side of Figure 1a, and the intersection with the curve represented by the broken lines. At pH 5.0, at the lower sodium concentration of 0.17 g sodium ions per liter, (curve "B"), the resin takes up about 0.4 mg-equivalent sodium ions/gm of dry resin. Thus, at pH 5.0, a pH at which cytochrome

C is clearly bound, the resin can take up only about 4.55% of the maximum sodium ion uptake (0.4 mg-equivalent sodium ions/gm of dry resin divided by 8.8 mg-equivalent sodium ions/gm of dry resin times 100% = 4.55%). Therefore, given the sodium ion uptake data presented in Boardman, and contrary to the opinion evidence provided in the Becker Declaration, Boardman's Amberlite IRC 50 resin is about 4.55% ionized at pH 5.0.

In view of appellant's definition of "electrostatically uncharged" as meaning "less than 5% of the ionizable functionalities on the resin are charged" (specification, page 18), it is clear that the pH 5.0 complex between cytochrome c and Amberlite IRC 50 described by Boardman is in fact a complex between a solid support matrix having an ionizable ligand thereon and a protein, at a pH from 5 to 9, wherein the resin is electrostatically uncharged.

With respect to the argument regarding the claim limitation requiring the resin to be uncharged at high ionic strengths, it is noted that the specification at page 18 defines "high" ionic strength as greater than 250 millimolar. However, that limitation does not appear in the claims under examination. On the current record the term "high" can be construed to encompass any concentration which is higher than another. That concentration clearly appears in Boardman.

With respect to the argument that Boardman's Amberlite resin does not contain ionizable ligands, an argument apparently not presented previously, note specifically that the carboxyl moiety on the resin is in fact ionizable, and can be used to adsorb ions, as evidenced by the sodium adsorption data in Boardman discussed above. Appellant's claims simply do not exclude resins having the ionizable ligand as part of the solid matrix. Similarly, the fact that Amberlite is not salt independent is not directly relevant because Amberlite meets all of the structural requirements recited in the claims.

With respect to the fact that claim 2 is allegedly not anticipated by Boardman, it is noted initially that this argument has not been presented previously. Moreover, even if Boardman does not anticipate claim 2, it is noted that claim 2 is included in the rejection under § 103(a) set forth herein. With respect to the fact that claim 5 requires a "spacer arm" between the ionizable ligand and the support matrix, note that the methyl group of the methacrylic acid can be considered a spacer arm. In sum, the pH 5.0 complex between cytochrome c and Amberlite IRC 50 described by Boardman is in fact a complex between a solid support matrix having an ionizable ligand thereon and a protein, at a pH from 5 to 9, wherein the resin is

electrostatically uncharged. The anticipation rejection over Boardman should therefore be maintained.

With respect to the issue of obviousness appellant initially urges that the Sasaki references, taken by themselves, have significant deficiencies. However, the Sasaki references are applied in combination with a number of other references, and it is therefore improper to consider the Sasaki references without consideration of the disclosures of the other references. Thus, in response to appellant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

Appellant further argues that there is no motivation to modify the Boardman or Sasaki references to arrive at the claimed invention. With respect to Boardman, appellant urges that the poor yields fail to suggest the desirability of the claimed invention. However, it is respectfully submitted that appellant's claims do not recite any limitation with respect to yield. Thus, appellant's claims are not distinguishable from Boardman's disclosure on the basis of yield. Moreover, to the extent that appellant suggests that producing the claimed

complex ultimately produces an unexpectedly high yield of the protein or peptide, appellant points to no direct evidence demonstrating any yield beyond that obtained in Boardman.

Appellant further urges that the Sasaki disclosures are limited to acid-stable proteins/peptides, and that taken together, the Sasaki references and Boardman suggest forming resin-protein/peptide complexes at a pH of 4.5 or below, a pH lower than the claimed pH of 5 to 9. However, the Sasaki et al. references were cited because they clearly disclose the concept of the instant invention. The cartoon of Figure 5 in Sasaki et al. (1982) outlines the method in its most general form. The cartoon illustrates an acidic group undergoing ionization. At low pH the proteins absorb to the uncharged resin by hydrophobic effects. As the pH is raised, the resin becomes charged and the proteins elute.

With respect to using the general concepts discussed in Sasaki within the pH range recited in appellant's claims, the legend of Figure 5 of Sasaki (1982) goes on to say that the method "can be used with an absorbent carrying alkaline groups, although the relationship to pH would be the opposite". That is, at high pH the resin is uncharged and the proteins bind by hydrophobic effects. As the pH is lowered, the resin becomes charged and the proteins elute. Sasaki et al. only illustrates

the method with a resin that binds at pH 4.5. It is clear that their conceptualization only requires that there be resins which bind in the critical region of 5-9.

Thus, the Sasaki references provide the artisan of ordinary skill with a reasonable expectation that the processes exemplified therein would have been suitably applied to purification of proteins within the pH range recited in appellant's claims. Sasaki provides a general concept and all of these three references (Sasaki (1979), Sasaki (1982), and Boardman) merely provide specific examples. As is well known, the examples are non-limiting to the concept. They only provide working evidence of concept, unless there is some reason to believe the examples are the only possibility. In the instant case, the explanatory cartoon in Sasaki belies that suggestion. No specific pH values are given. Boardman is simply a specific example.

The Kunin, Topp, Kitchener and Guthrie references make it clear that chromatographic media having pKa values within the claimed pH range were known in the art, and that the pKa values of those resins was readily determined by titration. The remaining references are cited to make it clear that resins having the specific ionizable moieties recited in the claims were in fact well known to the artisan of ordinary skill, this

shortcoming of the original *prima facie* case of obviousness being pointed out in the original Board decision as being unsupported by citation to a prior art document.

In sum, on the current record, the claims are directed to the formation of a resin-protein/peptide complex at a pH which the cited prior art contemplated as being suitable for the purification of proteins, given suitable chromatographic resins. See Sasaki (1982), page 1560, Fig. 5, legend. ("Hydrophobic-ionic chromatography can be carried out with the use of an appropriate adsorbent carrying alkaline groups, although the relationship to pH would be opposite.") As evidenced by the cited prior art, adsorbent resins possessing alkaline groups were generally known in the art at the time of appellant's invention (see Kunin, Topp, Kitchener and Guthrie), as were adsorbent resins possessing the specifically claimed ionizable groups (see Hancock, Kitamura, Tokuyama, Kondo, Iimuro, Bruegger, Economy, Jones). Thus, solely by following the suggestion in the prior art, one of ordinary skill in the art at the time of appellant's invention would have been motivated to have practiced the invention recited in appellant's claims.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

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FCP
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